INTRODUCTION

Increase in food production is the prime-most objective of all countries, as world population is expected to grow to nearly 10 billion by 2050 (Saravi and Shokrzadeh 2011; Kaur and Garg, 2014). The Food and Agricultural Organization (FAO) of the United Nations has issued a sobering forecast that world food production needs to increase by 70%, in order to keep pace with the demand of growing population. Increase in food production is experiencing the ever-growing challenge especially the new area that can be increased for cultivation purpose as forests and cultivation lands are being utilized for abode construction leading to a deteriorating environmental imbalance (Ullah, 2014). The increasing world population has therefore put a tremendous amount of pressure on the existing agricultural system so that food needs can be met from the same current resources like land, water etc. In the process of increasing crop production, agrochemicals in the form of herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments are now being used in higher quantities than in the past. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced. The rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played chaos with human and other life forms (Aktar *et al.,* 2010). These chemicals have mainly come into the picture since the introduction of synthetic insecticides in 1940, when organochlorine (OCl) insecticides were first used for pest management. Before this introduction, most weeds, pests, insects and diseases were controlled using sustainable practices such as cultural, mechanical, and physical control strategies.

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Among these, organochlorine (OC) insecticides which were used successfully in controlling a number of diseases, such as malaria and typhus, were banned after the 1960s in most of the technologically advanced countries. The introduction of other synthetic insecticides – organophosphate (OP) in the 1960s, carbamates in 1970s and pyrethroids in 1980s as well as the introduction of herbicides and fungicides in the 1970s–1980s contributed greatly to pest control and agricultural output. The use of agrochemicals is necessary to control pests and increase yields in order to produce adequate food for the global increasing (Jurado *et al*., 2011). The agricultural sector’s annual application of over 140 billion kilograms of fertilizers and large amounts of pesticides creates massive sources of diffuse pollution of freshwater systems through agricultural run-off (Schwarenbach, 2006; Sadekarpawar 2015; Patel *et al*., 2016). The presence of these toxic chemicals in aquatic and terrestrial ecosystems has become an important issue globally. Growing research-based evidence shows that agrochemicals, metals and industrial chemicals interfere with the health and normal functioning of the endocrine systems and overall physiology of a wide range of organisms, including humans (LeBlanc, 2007; Correia *et al*., 2010; and Pedersan *et al*., 2010; Abedi *et al.,* 2013).

It is believed that effects of these chemicals on the normal functioning of the endocrine system are responsible for a number of functional anomalies in a wide range of species, from invertebrates to higher mammals (Abel, 2002; London *et al*., 2005; Mihaich *et al*., 2009 and Benstead *et al*., 2011). The effect of agrochemicals in general and pesticides in particular on non-target organisms has been a source of worldwide attention and concern for decades. We live in a world in which man-made chemicals have become a part of everyday life. It is clear that some of these chemical pollutants can affect the endocrine (hormonal) system, and interfere with the developmental processes of humans and wildlife species (WHO, 2013). There are different ways by which pesticides can get into water such as accidental spillage, industrial effluent, surface run off and transport from pesticide treated soils, washing of spray equipments after spray operation, drift into ponds, lakes, streams and river water, aerial spray to control water-inhibiting pests (Kamble and Muley, 2000, Bhachandra *et al*., 2001; Madhab Prasad *et al*., 2002 and Sindhe *et al*., 2007;Singh and Mandal, 2013 and Larson *et al*., 2010).

Fishes are an important part of aquatic ecosystem as they interact closely with physical, biological and chemical environment. Fishes provide food source for other animals such as birds, mammals and thus form an integral part of the food web. A lot of research has been carried out to examine the impact of pesticides on different aspects such as changes in fish behaviour (Ilavazhahan *et al.*, 2010; Sadekarpawar and Parikh 2010; Nagaraju *et al.,* 2011; Prashanth *et al.*, 2011; David *et al.,* 2012; Desai and Parikh, 2012; Satyavardhan 2013; Ullah *et al.,* 2014; Rani and Kumaraguru, 2014), haematological changes (Gautam and Kumar, 2008; Devi *et al.*, 2008; Saeedi *et al.*, 2012; Desai and Parikh 2012; Upadhyay *et al.*, 2014), histopathological alterations (Johal *et al.*, 2007; Kunjamma *et al.*, 2008; Velmurugan *et al.*, 2009; Desai and Parikh., 2012;

Ba-Omar *et al.,* 2011; Rani and Venkataramana, 2012; Deka and Mahanta, 2012; David and Kartheek, 2014; Patel *et al*., 2016), biochemical modifications and enzymes alteration (Çavas and Könen, 2007; Firat *et al*., 2011, Scholz *et al*., 2012; Desai and Parikh 2013; Murthy *et al*., 2013; Dey and Saha, 2014; Sadekarpawar *et al.*, 2015a & b) and changes in antioxidant defence system (Shanker *et al.,* 2005; Milaeva, 2006; Neto *et al.*, 2008; Nwani *et al.,* 2010; Cao, *et al.*, 2010; Sevcikova *et al.*, 2011; Muthukumaravel *et al.*, 2013 Desai and Parikh 2013; Sadekarpawar *et al.*, 2015; Patel *et al*., 2016).

Organophosphate pesticide "Abate" has been proved to alter the vitellogenesis of catfish (*Heteropneustes fossilis*- Bloch.), which then severely affect catfish farming (Kumari, 2012). Olfaction conveys critical environmental information to fishes, enabling activities such as mating, locating food, discriminating kin, avoiding predators and homing. Waterborne contaminant disrupts all of the olfactory based responses by multiple mechanisms: can act as signals, modify odorant perception, and/or act on the nervous system and/or other physiologic response, all of which potentially alter normal olfactory mediated responses. As reported by Tiemey and co-workers (2010) contaminants mimic naturally-occurring odorants which change the stream chemistry thus becoming biologically unavailable. They may also disrupt the endocrinology of fish, thereby causing them to send situationally inappropriate cues. Similarly, glyphosate exposure to trematode parasite (*Telogaster opisthorchis*), freshwater fish (*Galaxias anomalus*) and snails has been reported to significantly reduce their survival and development (Kelly *et al.,* 2010).

Agrochemicals not only impact the fish physiology but also food webs related to them. The persistent pesticides (organochlorine pesticides and polychlorinated biphenyls) have been found in the major Arctic Ocean food webs (Hargrave *et al*., 1992). The impact of agrochemicals within an aquatic environment is influenced by their water solubility and uptake ability within an organism (Pereira *et al*., 2013). For example, Clomazone, a popular herbicide, is water soluble; a property that increases its likelihood of contaminating surface and groundwater. The hydrophilic (water-loving) or lipophobic (fat-hating) nature of this pesticide makes it less available in the fatty tissues of an organism (Pereira *et al*., 2013). Further to this, the toxicity of chemical (e.g., endosulfan) in juvenile rainbow trout (*Oncorhynchus mykiss*) was affected by alkalinity, temperature of water and size of the fish (Capkin *et al.,* 2006). Pesticides in natural water within the acceptable concentration range also pose harmful effects as reported by Kock-Schulmeyer *et al.,* (2012) and has suggested that proper measures should be taken while disposing of expired pesticides as their discharge into the water bodies change the water quality and the alteration in water pH by expired insecticides can lead to acute toxicity of different fish (Satyavani *et al.*, 2011).

Generally, agrochemicals which act as EDCs can work through three mechanisms of action: (i) Agonistic/antagonistic effect ('hormone mimics'), (ii) Disruption of production, transport, metabolism or secretion of natural hormones, and (iii) Disruption of production and/or function of hormone receptors (Goksoyr *et al.,* 2003; Rotchell and Ostrander 2003). This classification indicates discrete and separate routes of action of an EDC, depending on the properties of the compound responsible. Studies on estrogenic activities of EDCs have been carried out to monitor the reproductive fecundity for e.g. hermaphroditism and estrogenic responses in rainbow trout (Thorpe *et al*., 2008; Harris *et al*., 2001 and Tyler *et al*., 2002); increased blue sac disease and early life stage mortality in Great Lakes salmonid populations (Mcmaster , 2001; Ullah and Zorriehzahra 2015); alterations in endocrine homeostasis and reproductive fitness such as reduced gonad size, egg size and fecundity and delayed sexual maturity (Hoeger *et al.,* 2005; Zorriehzahra, 2008); depression of plasma sex steroid hormone levels and inhibition of gonadal development (Singh *et al.,* 2010). While many of these responses have been recognized for more than a decade, uncertainty remains with respect to the mechanisms by which these agrochemicals affect reproduction and development. In certain cases, the identity of the responsible chemicals remains unknown; also, when endocrine disruption has been noted, it is not know if this is primarily due to disruption of endocrine homeostasis or if it is the result of other mechanisms of toxicity (Ankley *et al*., 2009).

There is considerable homology between the endocrine systems of fish and higher vertebrates with respect to the nature of the hormones, their receptors, intracellular signaling pathways mediating hormone action, and in the regulation of the endocrine system (Papoutsoglou 2012). However, there are also subtle differences in the endocrinology of fish, as seen in the levels of circulating androgens which differs between classes (Yaron *et al*., 2003). In some fish, 11-oxygenated androgens, especially 11-ketotestosterone, are present in high quantities in blood of fish and represents the biologically active androgen responsible for stimulating secondary sex characteristics, reproductive behavior and spermatogenesis (Johnston 2013).

Even though there appears to be more examples of endocrine disruption in fish compared to other wildlife groups, there is little evidence to support the idea that fish are actually more sensitive to the effects of agrochemicals than other wildlife. The widespread endocrine disruption observed in fish relative to other wildlife may be due to their increased exposure to these chemicals as a result of their aqueous environment coupled with aspects of their physiology that create additional routes of exposure as both aquatic respiration and osmoregulation also contribute to the increased bioavailability of agrochemicals (Lyssimachou *et al*., 2008).

Cortisol

Cortisol, as a stress responsive hormone under toxic conditions, has been widely reported in a number of fish under different types of pesticide exposure. Exposure of fish to different types of pesticides showed alterations in the serum cortisol levels in fish such as exposure of *Oncorhynchus mykiss* to DDT compounds (Benguira and Hontela, 2000), flat fishes to chlorinated hydrocarbons (Schiff and Allen, 2000) and *Salmo gairdneri* to carbaryl (Balow and Rosenthal, 2001) and to endosulfan (Leblond *et al*., 2001) caused different levels of changes in serum cortisol as a stress response. Similar patterns of results were also reported in *Oncorhynchus mykiss* exposed to o,p’ -DDD,p,p’ -DDT and p,p’ -DDD compounds (Benguira and hontela 2000). Reduction in cortisol secretion by adrenocortical cells of rainbow trout, *Oncorhynchus mykiss* exposed to endosulfan was reported by Dorval and Hontela (2003) , Dorval *et al*. (2003). Jasmine Christal *et al*. (2003) reported changes in cortisol levels in *Sarotherodon mossambicus* exposed to dimecron and cuman L.

Changes in the serum cortisol levels of *Oreochromis mossambicus* exposed to endosulfan were reported by Parvatham *et al*. (2004). Lacroix and Hontela (2004) reported disrupted cortisol synthesis during adrenosteroidogenesis in rainbow trout, *Oncorhynchus mykiss* towards acute exposure of o,p’- DDD compounds. Sumathiral (2006), while studying the endocrine responsiveness in a freshwater air-breathing teleost, *Channa striatus* exposed to sevin, reported significant reductions in serum cortisol levels both under lethal and sub-lethal exposures. Disruption in the cortisol secretion was reported in rainbow trout, *Oncorhynchus mykiss* exposed to copper (Gagnon *et al*., 2006) and to cadmium (Gagnon *et al*., 2007). Hadi *et al*. (2009) reported significant increase in the serum cortisol levels in Tilapia *zilli* exposed to aluminum. Thangavel *et al*. (2010), while studying the hormone profile of an edible freshwater teleost, *Sarotherodon mossambicus* under endosulfan toxicity, reported significant changes in the serum cortisol levels for different periods of exposures. Elevated levels in the serum cortisol were reported by Zaki *et al*. (2010) in Tilapia *zilli* under lead pollution. Maheswari (2010) reported comparative changes in the serum cortisol levels of *Sarotherodon mossambicus* exposed to three different groups of pesticides.

Thyroid stimulating hormone (TSH)

Compared to investigations on the serum cortisol levels in fish toxicology, effects of pesticides on thyroid stimulating hormone (TSH) in fish have been investigated only by a few researchers. Reduced serum TSH levels were reported in *Heteropneustes fossilis* exposed to safe and sub-lethal concentrations of cythion and hexadrin (Singh and Singh, 1980). Severe reduction in TSH hormone levels indicating reduced thyroid function and reduced metabolic activity in *Salmo salar* were reported by Freeman and Sangalong (2000). Significant changes in the serum TSH levels of *Cyprinus carpio* exposed to sub-lethal endosulfan as a stress response was reported by Jenkins *et al*., (2003). Parameshwari *et al*. (2004) reported significant changes in the serum levels of TSH in *Sarotherodon mossambicus* exposed to individual and synergistic concentrations of dimecron and cuman L. Sumathiral (2006) reported variable magnitudes of changes in serum TSH levels of *Channa striatus* exposed to lethal and sub-lethal concentrations of sevin. Saravanan *et al*., (2010) reported reduced levels of TSH in *Labeo rohita* exposed to endosulfan. *Sarotherodon mossambicus* exposed to three different groups of pesticides registered different magnitudes of changes in the serum TSH level (Maheswari, 2010).

Triiodothyronine (T_3) and thyroxine (T_4)

In fish, hormones are critical towards maintaining proper physiological function and amongst the many hormones found in fish the thyroid hormones (thyroxine-T_4 and triiodothyronine-T_3) are known to play an important role in fish growth (Higgs *et al*., 1982; Miwa and Inui, 1985) and early development (Brown, 1997). When fish are exposed to stressors the levels of thyroid hormones have been demonstrated to be decreased (Pickering, 1993; Deane *et al*., 2001) and chemical pollutants have been reported to detrimentally affect thyroidal hormone status in a number of fish species (Xu *et al*., 2002; Brown *et al*., 2004; Scott and Sloman, 2004; Van der Ven *et al*., 2006).

Brown *et al*., (1984) reported significant changes in the serum thyroid hormone levels of rainbow trout, *Salmo gairdneri* exposed to acid and lime water. Similar changes were reported in the freshwater catfish, *Heteropneustes fossilis* exposed to malathion and BHC (Yadav and Singh, 1986). Exposure of *Channa punctatus* to different industrial effluents caused changes in thyroid function of the fish (Bhattacharya *et al*.,1989). Sinha *et al*.(1991) reported carbaryl induced thyroid dysfunction in freshwater catfish, *Clarias batrachus*. *Anabas testudineus* exposed to a detergent, teepol registered significant changes in serum levels of T_3 and T_4 (Leji and Subash Peter, 2003). *Channa punctatus* registered significant changes in thyroid function when exposed to different concentrations of metacid-50 and carbaryl (Ghosh *et al*.,1989). Malathion induced changes in serum thyroxine levels were reported in *Heteropneustes fossilis* (Nath *et al*.,1996). Peter and Anand (2003) reported changes in serum T_3 and T_4 levels in *Anabas testudineus* exposed to malathion; in *Oreochromis mossambicus* exposed to endosulfan (Parvatham *et al*., 2004) and in *Sarotherodon mossambicus* exposed to dimecron (Thangavel *et al*., 2005). Significant changes in serum thyroxine levels were also reported in *Sarotherodon mossambicus* exposed to individual and synergistic concentrations of dimecron and cuman L (Parameshwari *et al*., 2004). Significant reductions in T_3 hormone levels were reported in *Channa striatus* under lethal and sub-lethal exposures (Sumathiral, 2006) which was taken by the author to suggest as an adaptive response of fish towards reducing the basal metabolic rate. Brar *et al*. (2010) reported significant reduction in the plasma thyroxine (T_4) level in two indigenous fish species sampled from different polluted sites of San Francisco Bay.

Changes in the levels of T_3 and T_4 were also reported in endosulfan exposed *Sarotherodon mossambicus* for different periods (Thangavel *et al*.,2010). Maheswari (2010) investigated the serum levels of T_3 and T_4 hormones in *Sarotherodon mossambicus* exposed to sub-lethal concentration of sevin, endosulfan and dimecron. Significant elevations in T_3 levels were reported by Saravanan (2010) in *Labeo rohita* exposed to sub-lethal concentration of endosulfan. El-Khaldi (2010) reported reduced T_3 and T_4 levels in Nile Tilapia exposed to stress factors of hypoxia, overcrowding and starvation for 24, 72 and 144 hours. Hormonal and ion regulatory responses were studied in three different freshwater fish species following waterborne copper exposure (Eyckmansa *et al*., 2010).

Estrogens

Aquatic contaminants can compromise reproduction, development, immune response and other physiological processes, which can ultimately affect the survival of fish (Iwanowicz *et al*., 2011; Diamanti-Kandarakis *et al*., 2009 and Kloas *et al*., 2009). In addition to the direct impact of aquatic contaminants on fish populations, the ecological importance of fish means that they also indirectly affect the environment and, when eaten by humans and wildlife, pose a health risk and negatively impact the economics of fisheries and aquaculture. Estrogens are steroid hormones found in representatives of all classes of marine and terrestrial vertebrates including fish, amphibians, reptiles, birds and mammals. Many agrochemicals are known to have estrogenic effects as reported in many teleosts, *in vivo* and *in vitro* (Kolpin *et al*. 2002 Anderson *et al*., 2002; Kim *et al*., 2004; Xie *et al*. 2005; Lavado 2009; Scholz *et al*. 2012), mediated through interaction with the estrogen receptor α (ER α) (Okubo *et al*.,2004; McCarthy *et al*., 2006), by either mimicking or interfering with the actions of the natural ligand 17-β estradiol on the reproductive system (Brian *et al*. 2005; Correia *et al*. 2007; Zhang *et al*. 2010). These ER-mediated actions of environmental estrogens include induction of vitellogenin and sexual differentiation. Experiments with minnows suggest that exposure to environmental estrogens may cause problems for fish populations, where severe levels of feminization has been reported with occurrence of egg cells in the testes which can impair sperm quality, impede fish's ability to reproduce (Crago *et al*., 2012).

Androgens

The majority of toxicological studies conducted to date have focused on agrochemicals modulating the estrogenic response system in one way or the other (Urbatzka *et al*., 2007a & 2007b; Kloas *et al*., 2009; Behrends *et al*., 2010). In contrast to the intensive research focused on man-made chemicals that potentially disrupt normal estrogenic function, relatively few studies have addressed interaction with the androgenic endocrine systems, with an agonistic or antagonistic perspective (Urbatzka *et al*., 2007a). Chemicals modulating the androgen receptor (AR), resulting in an anti-androgenic response, have made known to adversely affect male reproductive health, causing testicular dysgenesis syndrome, cryptorchydism, testicular cancer (Skakkebsk *et al*., 2001; Kortenkamp and Faust, 2010; Orton *et al*., 2011). Chemicals having AR antagonistic properties are of specific concern, as these contaminants can compete with androgens to bind to the AR, resulting in decreased transcription of target genes (Birkhoj *et al*., 2004; Ermler *et al*., 2010, 2011).

Scientists have long blamed environmental estrogens in wastewater for feminizing male fish downstream of sewage plants. However, a recent study (Bertram *et al*., 2015) of treated wastewater identifies a wide range of antiandrogens compounds that block accumulation of male hormones in fish. Further, researchers from Monash University (Bertram *et al*., 2015) in collaboration with researchers from Åbo Akademi University in Finland (Saaristo, 2015), have established that the steroid 17β-trenbolone used on livestock to increase muscle growth alters male reproductive behavior in guppy fish (*Poecilia reticulata*) with androgenic and anabolic potency 15–50 times greater than testosterone (Morthorst *et al*., 2010; Gall *et al*., 2011; Kolodziej *et al*., 2013).

In vitro profiling of the endocrine disrupting potency of organochlorine pesticides has also been reported by Li *et al*., (2008) and has opined that it entails anti-agonistic effects. Further, it has been reported that Cyp17, the enzyme responsible for conversion of progesterone to androstenedione (Blystone *et al*., 2007) and Cyp19 (aromatase), which converts testosterone to 17β-estradiol (E2) (Hinfray *et al*., 2006) is also able to antagonize androgen receptor (AR) and estrogen receptor (ER) activity (Andersen *et al*., 2002). Together the inhibition of steroidogenic Cyp enzymes and antagonism of steroid hormone receptor activity results in altered levels of the circulating sex hormones testosterone (T) and 17β-estradiol (E2). PCZ-dependent depression of plasma T and E2 levels has been earlier observed in various fish models (Ankley *et al*., 2005 and Ankley *et al*., 2009b) and these alterations were linked to reduced reproductive success (Zhang *et al*., 2008).In general, these compounds act as sex hormones' blockers, which leads to anomalous and. Susceptible fish reproductive behaviour depicts its vulnerability by different pollutants such as pesticides (Hoeger *et al*., 2005) can also alter other hormonal processes of fish like development of bones and proper thyroid functioning (Murthy *et al*., 2013).

The changes in the endocrine function could arise through interference of the substance with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development and/or behavior. The endocrine system has a fundamental role in the regulation of endogenous metabolic processes in an organism. It coordinates the function between different organs through hormones that are released in the blood stream from specific types of cells within endocrine glands, Neurological, behavioral and reproductive processes are regulated by the endocrine system, as are growth, organ functions and responses to all forms of stress. These endocrine control mechanisms have been widely conserved among animal phyla. Disorders in any of these mechanisms with consequent alterations in hormone secretion will result in dysfunction, affecting different organ, and are then delibilitating or life-threatening. Therefore, the threat posed from agrochemicals with endocrine activity is potentially serious (WHO, 2002). ***Hence, the present study aims to measure the hormonal status of O.mossambicus exposed to agrochemicals (Chapter I).***

The genomic action of hormones is mediated by their binding to specific hormone receptors expressed in the target tissues. Such interactions lead to an increase in the production of intracellular second messengers, which are more directly responsible for the activation of the cell and the regulation of physiological processes (Nikolenko and Krasnov, 2007). The use of hormone cascades to regulate physiological processes is a strategy that has been conserved during evolution. This cascade provides a communication link between the nervous system and endocrine system in animal phyla. Teleost fish appear to posses all the nuclear receptor types found in mammals. Due to an apparent whole-genome duplication that causes the expansion of a large number of gene families in the teleosts lineage, followed by the retention of some duplicates and the loss of others, teleost fish have more nuclear receptors than most mammals (Thornton, 2003).

The anatomical part of fish brain has four major divisions (with, however, numerous differentiations among studied individuals of fish groups, e.g. Families, Genus, Species) that have so far been detected in adult individuals (Northcutt, 2008, Braford, 2009, Burmeister *et al*., 2008, Mueller and Wullimann, 2009, LaDage, *et al,* 2009, Rodriquez-Moldes, 2009, Gonzalez and Northcutt, 2009) are the following: a) the Forebrain/Cerebrum containing the Telencephalon and the Diencephalon, b) Mid-brain/Mesencephalon, and c) the Hind-brain/Rhombencephalon, containing the Metencephalon and Myelencephalon/medula oblongata. Further, according to

Papoutsoglou (2012) the fish brain is classified into Superior brain centers which include olfactory bulb, Epiphysis, Optic tectum, Pallium, and sub-cortical regions Amygdala, Hippocampus, Cerebellum, Thalamus, Hypothalamus and Pituitary-Hypophysis**.** Hypothalamus a hormonally autonomous gland is situated in the lower part of brain (base), having two ends, one end connected to pituitary and other end connected to thalamus.

The hypothalamic neurohormones, which promote the production and secretion of the pituitary hormones, are known as releasing hormones or liberins (Guillemin 2005). Hypothalamus is composed of three areas (groups of cells) that are characterised by the phenomenon of neurosecretion. These areas are called nuclei a) the preoptic nucleus or nucleus preopticus b) the lateral nucleus or nucleus lateralis tuberalis c) the paraventricular organ or saccus vasculosus, which is complex and consists of the nucleus recessus lateralis (NRL) and the nucleus recessus posterioris (NRP). In most fish, the pituitary gland is linked to the hypothalamus by the pituitary stalk. There are basically two types of connection between the pituitary gland and the hypothalamus in fish. One is the open or wide type where the pituitary stalk is rather broad (e.g. *A. anguilla*, *Salmo salar*). In all cases of this type, tissue from the inferior portion of the hypothalamus (infundibulum hypothalami) reaches the pituitary gland through the stalk. The pituitary stalk, which in certain species (e.g. *Lophius piscatorius*) is particularly apparent, consists of neuroendocrine cell axons which originate in the hypothalamic nuclei. The other is the closed type where the stalk is not physically connected to the pituitary gland and thus does not include any hypothalamic tissue. The pituitary gland is divided into three main parts: the anterior pituitary, or adenohypophysis, the intermediate lobe; and the posterior pituitary, or neurohypophysis. As the study revolves around the three main axis HPG, HPI, HPT and it hormones are governed by the action of anterior pituitary thus anterior pituitary is taken into account. The teleost anterior pituitary consists of two portions (containing at least six different cell types), the **rostral** *pars distalis* and the **proximal** *pars distalis*. The rostral *pars distalis* includes η, ε, δ cells and the proximal *pars distalis has* α, β, γ, cells (Figure I).

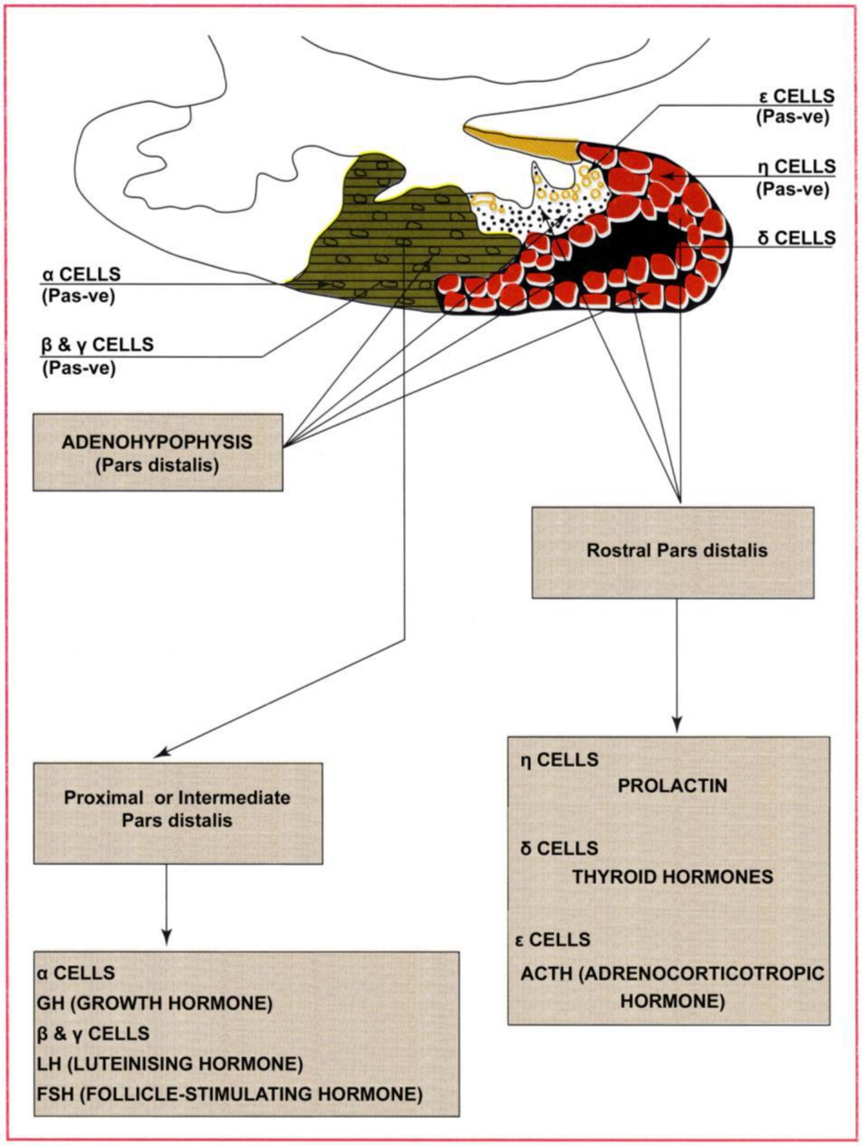

Figure I. Schematic representation illustrating the anatomical structure of the anterior pituitary (adenohypophysis) of the teleost pituitary gland (Papoutsoglou 2012).

To understand the toxicity and alteration caused by the agrochemicals, the study is focused on three axis; hypothalamo pituitary gonadal axis (HPG), hypothalamo pituitary interrenal axis (HPI), hypothalamo pituitary thyroid axis (HPT).

Hypothalamo Pituitary Gonadal Axis (HPG):

Gonadotropin releasing Hormone (GnRH):

Reproduction in all vertebrates is controlled by the highly conserved HPG axis. At its apex are the GnRH neurons in the hypothalamic-preoptic area of the brain that ultimately control reproduction by integrating information from social and environmental signals with internal information such as nutritional and hormonal state. (Holland *et al.*, 2001). There are three different forms of GnRH hormone in most fish (Carolsfeld *et al.*, 2000) each with a single short polypeptide coded by a distinct gene. The gene is located on chromosome no. 12 (figure II). They are termed salmon type or Trp 7 Leu 8 form, chicken II type or Trp 7 Tyr 8 form, and sea bream type or Ser 8 form, and each is synthesized in distinct regions of the brain. The *GnRH* genes of vertebrates share a common structure, being by the presence of 4 exons and 3 introns. Sequence analyses of the different *GnRH* genes showed that coding regions are highly conserved, but upstream and downstream regions and intron sequences are distinctively divergent (Zohar *et al.,* 2010).

The decapeptide GnRH issues from a large mRNA. The initial mRNA product, a precursor peptide called prepro-GnRH, consists of a signal peptide that allows the protein to be transferred to the endoplasmic reticulum, the mature GnRH decapeptide, a processing tripeptide (Gly-Lys-Arg), and a GnRH-associated peptide or GAP. The signal peptide, GnRH decapeptide, processing tripeptide and N-terminal region of GAP are encoded in exon 2. The core and C-terminal parts of GAP are encoded in exons 3 and 4. The 5′- and 3′-UTR are encoded in exons 1 and 4, respectively (Zohar *et al.*, 2010). In teleost, GnRH1 neurons in the preoptic area of the brain send axonal projections directly to the anterior pituitary gland where GnRH1 is released from nerve terminals in the vicinity of the gonadotrope cells, binds to membrane- bound G-protein-coupled GnRH receptors, and causes synthesis and release of the two gonadotropins, LH and FSH (Levavi-Sivan B *et al.*, 2010).

Analysis of the primary amino acids sequence of fish GnRH receptors indicates that they belong to the G protein coupled receptors family (GPCRs). Recent phylogenetic analysis of the GPCRs shows that they segregate into five subfamilies tentatively termed glutamate, secretin, adhesion, frizzled/TAS2, and rhodopsin subfamilies (Fredriksson *et al.,* 2003).

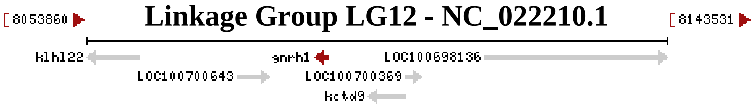

Figure II: GnRH I location on chromosome map.

A real-time fluorescence-based quantitative polymerase chain reaction study in the gilthead seabream demonstrated several concomitant elevations of mRNA levels of all three forms of GnRH, GnRH receptors, FSHβ, FSH receptor, LH receptor during the first 36 days post-fertilization (Wong *et al.,* 2004; Zohar *et al.*, 2010). However, new development in recent era has put another player in the picture that governing the GnRH release. ***The endocrine disrupting chemicals significantly alters the level of GnRH in the pituitary and other organs (Cheshenko et al. 2008; Page et al., 2011), but the toxicity of selected agrochemicals is yet to be tested on it. So, gene expression of GnRH-I was taken into account for this study (chapter II).***

Kisspeptin (KISS):

Kisspeptin was discovered as the metastasis suppressor gene having the sequence "KI" with Prefix "SS". Kiss 1and 2 is known o be located on 11th and 15th chromosome in teleost (figure III and IV). Different groups have identified four kisspeptin (Table 1) as a high-affinity RFamide (Arg-Phe-NH2) peptide ligand for a then orphan G protein-coupled membrane receptor, GPR54. Compelling evidence has now demonstrated the presence of two distinct genes encoding kisspeptins (*kiss1* and *kiss2*), and up to four different genes encoding their cognate receptors, *gpr54*s, in non-mammalian vertebrates, such as amphibians, reptiles and, preferentially, bony fish (Carrillo *et al.*, 2009; Lee *et al.*, 2009; Akazome *et al.,* 2010).

Studies carried out using genome and cDNA analyses of kisspeptin genes, revealed the similar structural organization of kiss1 and kiss2 genes i.e containing two coding exons with exon 2 coding for kisspeptin 2 (YNWNSFGLRY for kiss1 and FNFNPFGLRF for kiss2) (Felip *et al.*, 2009; Yang *et al.,* 2010;Zohar *et al.*, 2010). Teleost show high range of versatility of kiss genes. Studies have shown that almost all the species of it posses kiss2 gene, while kiss1 gene is also found in the genome of zebrafish, medaka, sea bass, goldfish and chub mackerel (Van Aerle, R. *et al*. 2008; Um *et al.*, 2010). On the other hand it is noteworthy that many species like *Takifugu rubripes* (tiger puffer), *Tetraodon nigroviridis* (green puffer) and *Gasterosteus aculeatus* (stickleback), lack the *kiss1* gene and possess only *kiss2* (Felip *et al.*, 2009; Kitahashi *et al.*, 2009; Yang *et al.*, 2010). ***However till now no authentic reports have proved the effect of***

toxicants on kisspeptin. Therefore this arouses an interest to look into the adverse effect of the selected agrochemicals on it and as a result kisspeptin was selected for the study (chapter II).

Linkage Group LG11 - NC_007122.6

Figure III: Kisspeptin 1 location on chromosome map.

Linkage Group LG15 - NC_022213.1

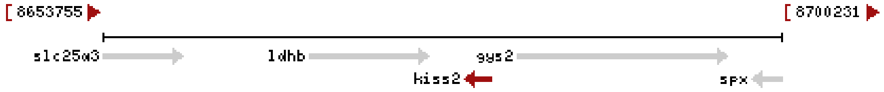

Figure IV: Kisspeptin 2 location on chromosome map.

Gonadotropins Receptors (GtHrs):

Gonadotropins (GtHs) are heterodimeric glycoproteins, sharing an identical α subunit and distinct β subunit which confers the biological specificity of the respective GtH. Based on the similarity of sequence specificity GtH Iβ was homologous to FSH β on the one hand, and GtH IIβ was homologous to LHβ on the other hand. Phylogenetic analyses based on amino acid sequences of vertebrate's gonadotropin subunits, derived from either chemically isolated peptides or deduced from cDNAs, revealed monophylogenetic lineage for each subunit (Quérat *et al*., 2000).Among all the vertebrates, GPα-subunit shows the highest degree of conservation which is around 60% to 90% due to the carboxyl-terminus half, which is the most conserved part of the molecule. Fish FSH β-subunits are more divergent than LH β-subunits (average identities of 53% and 67%, respectively) mainly due to a rapid change which occurred during the evolution of teleosts in general, and that of perciform species in particular (Kato *et al.*, 1993; Hassin *et al.*, 1995; Yaron *et al.*, 2003). Specifically, the N-terminal of teleosts FSHβ sequences exhibits an unexpected divergence at sites that are most conserved in FSHβ of other vertebrates, namely, asparagine (N) the potential glycosylation site, and cysteine (Cys).

The gonadotropins are known to bind to its receptor present on membrane of ovary and testis, these receptor were first established in early 1970 from binding studies (Breton *et al*., 1973;

Levavi-Sivan *et al.*, 2010). These receptors belongs to the family of rhodopsin- like receptors (family A), further they were termed as GtH Ir (FSh like) and GtH IIr (LH like) and follow canonical G-protein coupled receptor signaling mechanism (Gether, 2000). Together with the TSHRs they constitute the sub-family of glycoprotein hormone receptors (GpHR), whose general topology consists of a large extracellular domain followed by seven transmembrane helices and a carboxy-terminal intracellular tail (Vassart *et al.*, 2004). The gene of the receptors are known to be located on chromosome number 13 (figure V). Fish GtHRs follow this general structure, but the extracellular domains of fish FSHRs contain remarkable differences when compared to mammalian FSHRs (Oba *et al.*, 1999a, Oba *et al.*, 1999b ; Bogerd *et al.*, 2001;Kumar *et al.*, 2001b; Kumar *et al.*, 2001c; Vischer and Bogerd 2003 and Wong and Eenennaam, 2004; Kwok *et al.*, 2005; Maugars and Schmitz, 2006; Rocha *et al.*, 2007b; Sambroni *et al.*, 2007;).***However, data on the alteration in the receptor patterning exposed to any toxicant is very scarce, hence GtH Ir and GtH IIr was selected for the study (chapter II).***

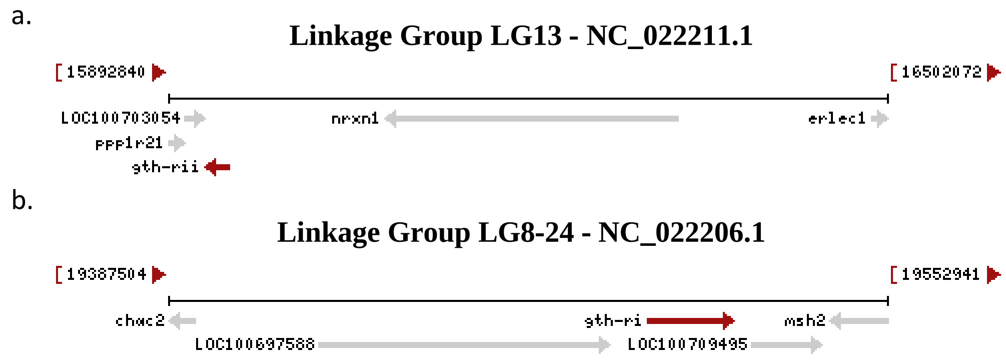

Figure V: Chromosomal map of GtH-IIR(a) and GtH-Ir(b).

Estrogen Receptors (ERs):

Steroid hormones are considered to be the one of the key player in maintaining the homeostasis, among them are the gonadal steroids which include estrogens (18 carbons), androgens (19 carbons) and progesterones (21 carbons) which are derivatives of the cyclo-pentanoperhydrophenanthrene ring from cholesterol. Estrogens are known to bind to its receptor known as estrogen receptor which belongs to nuclear receptor superfamily, a family containing receptors for small molecules (steroids, thyroid hormones, rexinoids, oxysterols, etc.) with a defined domain structure (Nelson and Habibi 2013).Similar to most nuclear receptors, ERs contain six distinct regions. The N-terminal region (NTD) is the most variable region between species. It contains the first activation function (AF1), which has been attributed to constitutive ligand-independent activation of the receptor (Weigel, 1996; Nelson and Habibi 2013). This region is known to be regulated by MAPK upon phosphorylation of it. N-terminal region is followed by the DNA binding domain (DBD), which is most highly conserved among all the species. The DBD contains two "zinc fingers" that folds together to form a compact 3-dimentional structure, allowing the receptor to interact with specific sequences of DNA referred to as hormone response elements, or in this case, estrogen response elements (EREs).In between the DBD and N terminal Domain (LBD) is the hinge region which often confers the nuclear localization signals. Following the hinge region is the LBD which is specifically known to bind to ligand and induces secondary activation function (AF2) located at the C-terminal region.

The gene of ER is found to be having linkage group on chromosome number 15 (figure V).Hawkins *et al.* (2000) have describe two isoforms /subtypes of ERs i.e. ER-I (ER α) and ER-II (ER β), where in some teleost the ER-II gene is found to be duplicated and has formed a variant of it.

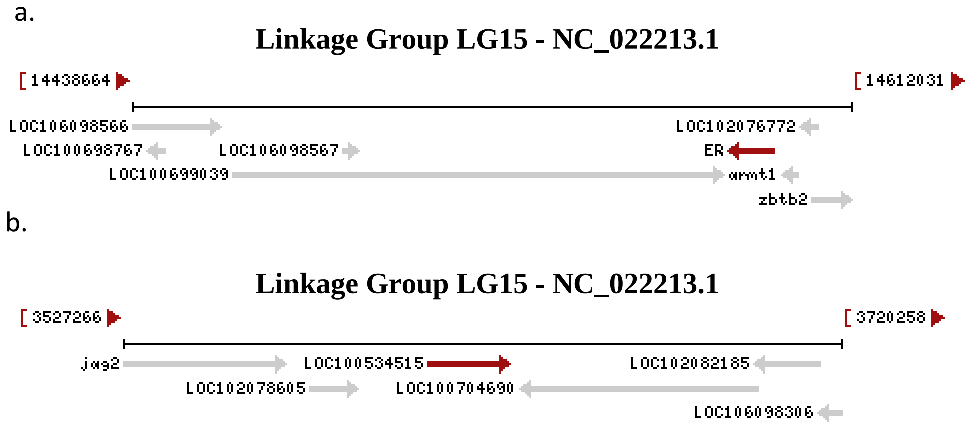

Figure V: Chromosomal map of ER-I(a) and ER-II(b).

In the present study, these receptors were selected as most of the pesticides act as endocrine disrupting chemicals (EDCs) the property which alters the mechanism of hormone and its signaling. The properties of present chemicals are not yet studied on ERs therefore the study was taken up (Chapter II).

Androgen Receptors (ARs):

The sex steroid signaling system has been shaped throughout the evolutionary history of vertebrates by two whole-genome duplication events, characterizing this lineage (Dehal and Boore 2005).The AR gene is known to have linkage group 2 as shown in figure VI and is a cytosolic protein that, upon fixation of a ligand, undergoes a conformational change and dissociates from chaperone proteins, dimerizes, and can translocate into the nucleus. The receptor subsequently binds to specific target sequences called androgen response elements and acts as a transcription factor together with coactivators or corepressors on specific target genes. It also belongs to the nuclear receptor superfamily, such as retinoid X receptor and thyroid hormone receptor, possess multiple subtypes and isoforms. ARs are composed of three main domains (Laudet and Gronemeyer 2002): a hypervariable N-terminal domain involved in transcriptio a. activation, a DNA binding domain (also referred to as the C Domain) which permits the binding of receptor on target genes and a ligand-binding domain (also referred to as the E Domain). The amino acid sequences of these last two domains (DBD and LBD) are highly conserved from actinopterygians to mammals with 90% and 70% identity with mammalian ARs for the DBD and the LBD respectively.

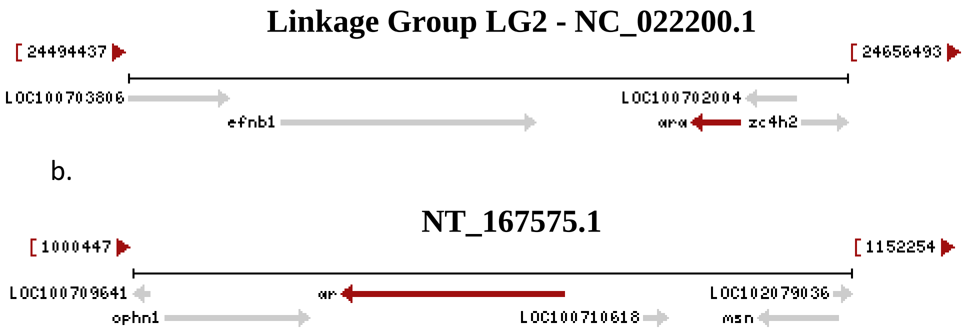

Figure VI: Chromosomal map of AR-I(a) and AR-II(b).

As most of the work done has proven the pollutants are xeno-estrogens, and less focused is being done on androgens. ***The effects of agrochemicals on the androgen receptors are less and remain erroneous and so, the ARs were selected for the study (Chapter II).***

Hypothalamo Pituitary Interrenal Axis (HPI)

Glucocorticoid and Glucocorticoid Receptor (GR):

Glucocorticoids are steroid hormones that are secreted by the adrenal gland after stress and in a circadian rhythm. Glucocorticoids in teleost fishes are involved in the regulation of a plethora of physiological functions, such as osmoregulation, respiration, immune responses, reproduction, growth, metabolism, and stress response (Mommsen *et al.*, 1999, Charmandari *et al.*, 2005). It is known to have linkage group LG2 (figure VIII) and belongs to the superfamily of nuclear receptors which is a modular protein consisting of four domains attributed with distinct functions. The amino-terminal A/B domain is important for transcriptional activity, the C-domain or DNA-binding domain (DBD) is responsible for DNA binding 3 Introduction and receptor dimerization, the D domain is involved in conformational changes, and the E-domain or ligand-binding domain (LBD) mediates hormone binding (Kumar and Thompson, 2005) as shown in figure VII.

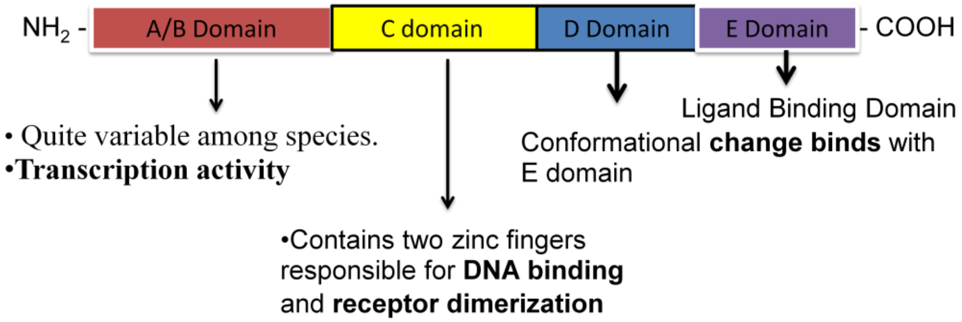

Figure VII: Structural and functional features of GR.

Recent studies demonstrated the presence of a two GR isoforms in various teleost species (Bury *et al.*, 2003; Greenwood *et al.*, 2003). ***The present work therefore set out to throw some light on the adverse effects of agrochemicals on glucocorticoid receptors and hormone cortisol of the teleostean corticosteroid system, with specific focus on O.mossambicus (Chapter I and II).***

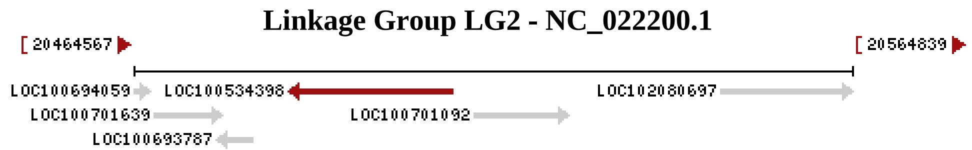

Figure VIII: Chromosomal map of GR.

Hypothalamic Pituitary Thyroid Axis (HPT):

Thyroid (THs) and Thyroid Stimulating Hormone (TSH):

In sub phylum vertebrata, the hypothalamic–pituitary–thyroid (HPT) axis regulates metabolism and growth, reproduction, and brain development. Correspondingly, in fish, thyroid hormones (THs) have been established to regulate growth (Power *et al*. 2001, Kang & Chang 2004), influence morphological development (Reddy & Lam 1992, Brown 1997, Tagawa & Aritaki 2005, Lema & Nevitt 2006), and mediate the changeover from larval or juvenile forms in a similar manner to how THs regulate metamorphosis in amphibians (Trijuno *et al*. 2002, Shiao and Hwang 2006).The thyroid hormone is produced from the thyroid follicles scattered in the pharynx region upon the stimulation of thyroid stimulating hormone (TSH). TSH is a heterodimer composed of alpha subunit and hormone specific beta subunit. These glycoprotein hormones act on their target tissues through their respective cell membrane receptors which are offenly known as thyroid stimulating hormone receptors (TSHRs). The gene of it is known to be located on LG15 (figure IX) and are 30 kDa dimeric proteins belonging to the glycoprotein hormone receptor family (GpHR) (Hsu *et al.*, 2000; Bhat *et al.*, 2015). GpHR belongs to the subclass of the large rhodopsin-like G protein-coupled receptors (GPCRs) family. The TSHR contain a large (300–400 amino acid) extracellular domain with at least 8 highly conserved Cys residues, involved in tertiary structure formation of extracellular domain that appears important in both ligand binding and in active receptor conformation (Vassart *et al.*, 2004; Bhat *et al.*, 2015).

Various studies have been carried out in relation to endocrine disrupting chemicals and TSH for e.g. reduced serum level were reported in *Heteropneustes fossilis* by Singh and Singh, 1979, reduced thyroid function and reduced metabolic activity in *Salmo salar* (Freeman and Sangalong 2000), changes in TSH levels *Cyprinus carpio* under the exposure of endosulfan, Jenkins *et al.*, 2003, magnitudes of changes in levels of TSH in *Channa striatus* exposed to sub lethal concentration of sevin (Sumathiral 2006) and magnitudes of changes in the serum TSH level in *Sarotherodon mossambicus* under the exposure of three groups of pesticides (Maheswari, 2010).

Linkage Group LG15 - NC_022213.1

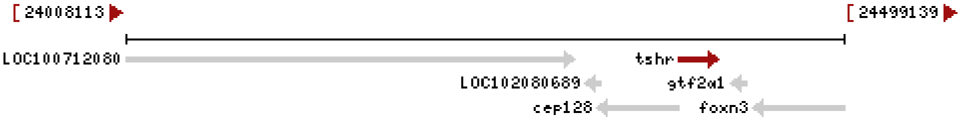

Figure IX: Chromosomal map of TSHr.

The group of agrochemicals selected for the study donot account to have reports on neither thyroid hormone nor on to its receptors. ***The present study is inclined to find whether the selected agrochemicals have any adverse effects on HPT or not. Therefore, the gene expression of TSHr (chapter II) and hormone profile was selected for the study (chapter I).***

Earlier studies carried out in our lab have proved the adverse effect of four agrochemicals on histological, hematological and biochemical alteration on two teleost *O.mossambicus* and *L.rohita*. However, there role in endocrine disrupting was lacking. ***Thus, in continuation with the previous studies Insecticide imidacloprid (IMI), fungicide curzate (CZ), herbicide pyrazonsulfuron ethyl (PE) and micronutrient mixture (MN) was selected for the study.***

IMIDACLOPRID

Over the last 2 decades, a new class of insecticides, the neonicotinoids, has become the most important and fastest growing of the five major chemical classes of insecticides on the global market (Jeschke and Nauen 2008; Jeschke *et al.,* 2011; Tomizawa and Casida 2011; Casida and Durkin 2013). When used as plant protection products, neonicotinoids act by becoming distributed systemically throughout the growing plant following seed or soil applications. Neonicotinoids work by interfering with neural transmission in the central nervous system. They bind to the nicotinic acetylcholine receptors (nAChR) in the postsynaptic neuron, acting as ‘false neurotransmitters’ (agonists). This interference with acetylcholine neurotransmitter signalling causes continuous activation of the receptor, leading to symptoms of neurotoxicity. Neonicotinoids have greater affinity and thus bind more strongly to, insect than mammalian or other vertebrate receptors, so their toxicity to mammals is lower than to insects (Tomizawa and Casida 2005; Jeschke *et al*. 2011). The insecticide imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine] (IMI) has been increasingly used since 1991 (Elbert *et al*., 1991) and belongs to the fastest growing group of insecticides introduced to the market, referred to as neonicotinoids (Tomizawa and Casida, 2003).

IMI is a potential groundwater and surface water contaminant (PAN Pesticides database, 2006), because it can leach and runoff from soil and crops (Felsot *et al.,* 1998; Gonzalez-Pradas *et al.,* 1999; Armbrust and Peeler, 2002; Gupta *et al.,* 2002; Fossen, 2006). Additionally, it may enter water bodies from spray drift or accidental spills, leading to local point-source contaminations.

Until now, the toxicity of IMI to aquatic invertebrates has been very few; this is due to the former belief that the compound is relatively immobile in soil and does not leach to groundwater (Bayer technical information for Confidor, 2000; Krohn and Hellpointner, 2002). In the case of low concentrations of chemicals, biochemical biomarkers are generally considered a more sensitive and sometimes more specific measure of toxic exposure. A review of literature and investigations on IMI toxicity on fish shows that a considerable work has been carried out as far as biochemical, histological and behavioral aspect is concerned (Padpa-priya *et al.,* 2012; Pdma-Priya and Maruthi, 2013; Rajput *et al.,* 2012; Desai *et al.*,2013; Parikh *et al.,* 2014 and Patel *et al.,* 2016).

Genotoxic Potential of the IMI has been studied in *Oreochromis niloticus* by Ansoar-Rodríguez, *et al.,* 2015 and have reported that the IMI induced primary DNA damage at the chromosomal level and proved the potential risk of IMI in a non-target organism. Due to the different mode of action of imidacloprid, an alternative strategy to the AChE bioassay should be found to warrant the investigation of imidacloprid-induced toxicity in nontarget species. To this extent, promising alternatives to traditional toxicity testing are found in the “omics” field (Martyniuk *et al*., 2011). Transcriptomics, proteomics, and metabolomics can measure changes in intracellular functioning upon exposure to toxicants at lower concentrations than traditional toxicity testing methods and can focus on numerous end points (genes, proteins, and metabolites) simultaneously (Waters and Fostel, 2004). The combination of these “omics” techniques in a system biology approach will enable a more accurate determination of the mechanism of action of toxicants, which may improve environmental risk assessment (Ankley *et al*., 2006; Van Aggelen *et al*., 2010). ***Hence, in the present study an attempt is made to report the Gene expression studies in fresh water cichlid, Oreochromis mossambicus (Chapter I and II).***

CURZATE (CZ)

CZ fungicide was discovered by Dupont and is primarily used on grapes, potatoes and tomatoes. It is currently registered for commercial use in over 50 countries on more than 15 crops. It is formulated as a 72% wettable powder: 8% cymoxanil and 64% Mancozeb.It is yellow coloured and odourless. Chemical name of the substance: Mancozeb is Manganese ethylenebisdithiocarbamate polymeric complex with zinc salt and that of Cymoxanil is 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea. Cymoxanil belongs to the class of aliphatic nitrogen fungicides. It acts as a foliar fungicide with protective and curative action. It has contact and local systemic activity, and also inhibits sporulation (FAO, 2005). It was first introduced in 1977, it is a compound used as both a curative and preventative foliar fungicide, as per FRAC (2012) it belongs to a chemical group cyano-acetamideoxime and has categorized it to be Low to medium risk as far as toxicity is concerned and have also suggested that resistance management is required. Cymoxanil provides effective control of economically important fungal plant pathogens, which cause downy mildew and blight in a wide range of crops. U.S.Environmental protection agency (1998) has classified Cymoxanil into Toxicity Category III for oral and dermal toxicity and Toxicity Category IV for inhalation toxicity with skin and eye irritation potential. The studies on cymoxanil have shown low oral acute toxicity on rabbits (Ponnana, 1999). The no- observable-effect-levels (NOEL) for chronic toxicity are reported in dogs, however, gross or histopathological effects were observed (Venugopala, 1999; Teunissen, 2003). The potential neurotoxicity of cymoxanil was evaluated in rats (Malleshappa, 2003) and mice (Krishnappa, 1999 a, b and 2002).

Cymoxanil is slightly toxic to fish and other estuarine and marine organisms on an acute basis (Baer, 1993 a and b; Kraemer, 1996). Mancozeb, another constituent of CZ M8 is an Inorganic-Zinc dithiocarbamate, is a typical fungicide with a carbamate structure where sulphurs replace both oxygens in the amide functional group. It is chemically identified as ethylenebisdithiocarbamate (EBDC). It is available in the form of powder with yellow colour and musty odor. The poisoning caused with EBDC compounds cause symptoms of irritation of skin, eyes and respiratory tract, skin sensitization; chronic skin disease has also been observed in occupationally exposed workers. Mancozeb is "moderately to highly toxic to fish and aquatic invertebrate animals," (Dupoint de Nemours, 1983). Mancozeb 80% WP is a fungicide that inhibits the production of thyroid hormones in vertebrates (Cocco, 2002). Mancozeb exposure is associated with pathomorphological changes in liver, brain and kidney. It has produced significant enzymatic changes in the activities of various enzymes in male rats (Kackar *et al*., 1999). Inhibition of implantation by Mancozeb due to hormonal imbalance or its toxic effects has been studied (Bindali and Kaliwal, 2002).

Thus, from the foregoing literature survey one can be summarize that CZ is a unique cyanoacetamide, chemically unrelated to any other commercial disease control agent and the biochemical mode of action is also different. The chemical has got systemic action for cymoxanil and moderate persistency for mancozeb (Roy *et al*., 2010). Because of its major metabolite ethylenethiourea, recently it has come under close scrutiny of health protection agencies due to its carcinogenic, teratogenic and goitrogenic effects in mammals (Das *et al.*, 2009). These studies suggest that Mancozeb and Cymoxanil have been individually studied in various animal models and found to be mild to moderately toxic. Bearing studies conducted by Desai and Parikh (2013. 2014 and 2015), where they have reported the biochemical, Behavioural and Histological alterations on exposure of Curzate, apart from this no studies have been recorded on CZ with reference to fresh water teleost fish. ***Hence, in the present study fungicide curzate, a combination of Cymoxanil and Mancozeb was selected. (Chapter I and II).***

MICRONUTIRIENT MIXTURE (MN):

Fertilizers containing trace elements (such as boron, copper, manganese, zinc, and cobalt) — in small quantities are called as micronutrient fertilizers. It is called micronutrients as they are needed only in minuscule amounts, these substances are the “magic wands” that enable the plants to produce enzymes, hormones and other substances essential for proper growth and development (Yoshida, 2008). Micronutrient fertilizers are specially formulated for delivering micronutrients with maximum bioavailability, tolerability, & safety. Micronutrients are available in two forms, chelated and nonchelated mixtures (Modaihsh, 1997). Most of the micronutrients are in chelated form, as they are absorbed quickly and easily by the crop thus providing effective organic nitrogen to overcome stress conditions and boosting up energy metabolism in the plant. Moreover it activates phytohormones and other growth substances in the crop increasing chlorophyll concentration and in turn boosting the photosynthetic activity maximizing growth and yield of crops like cotton, sugarcane, cereals, pulses, vegetables, fruit crops and floriculture. Micronutrient deficiencies are especially widespread in plants, animals, and human in many Asian countries due to the calcareous nature of soils, high pH, low organic matter, salt stress, continuous drought, high bicarbonates in irrigation water and imbalanced application of fertilizers (Sadekarpawar, 2014).

Indian agriculture is now in an era of multiple plant nutrient deficiencies. Nutrients like N, P, K, Zn, Mn, Mg, Mo, B, S and Cu are now of widespread practical importance from an application point of view. To meet this deficiency, application of trace elements in the form of fertilizers or micronutrients have been used rampantly whereas remediation of soils contaminated with metals is not addressed (Zhenli *et al.,* 2005). Repeated use of such metal-enriched chemicals, fertilizers, and organic moieties cause contamination of aquatic ecosystem by surface runoff leading toxic effect to non target organisms especially freshwater fishes. A frequently overlooked agrochemical is the plant nutrients added for biofortification of the soil. These nutrient supplementations, though enhancing food production, can have disastrous effects on the aquatic ecosystem as they readily leach out in the surface run off. A classical example of this condition is the death of alligators in Lake Griffin, Florida in 2000. The sudden deaths of alligators were caused by blooms of algae *Cylindrospermopsis*. Such algal blooms result due to leaching of various plant nutrients used in nearby farms. After this incident it was realized that not only pesticides, but plant nutrients can have detrimental effects on the environment. Unlike pesticides, which directly kill the organism/s, these plant nutrients may boost the growth of one organism and cause imbalance in the ecosystem leading to extinction of one or more species. Moreover, the studies conducted till date have been focused on the metal toxicity, the toxicity of the plant nutrient Librel TM on edible fishes has been thoroughly studied by Sadekarpawar *et al.,* (2013.2014.and 2015). ***However there is a lacunae as far as the gene expression studies are concerned and hence and attempt is made to have an insight into the molecular aspect for the same. (Chapter I and II)***

PYRAZONSULPHURONE ETHYL (PE)

Herbicides are the most commonly used pesticides, and are the most often detected in surface waters (Frans, 2004). Numerous commercial formulations containing different herbicides (glyphosate, paraquat, sulfonolurea etc) have become popular around the world due to their effective action and low toxicity to mammals (Corberaa *et al.,* 2005; Zhang *et al.,* 2010; Giovanni *et al.,* 2011). However; they have proved to be harmful to the environment. Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, pyrazosulfuron-ethyl herbicide is widely used for selective post-emergence control of annual and perennial grasses and broadleaved weeds in cereals. PE is widely used in rice crops in India (Singh *et al.,* 2012). There is limited information available on the fate of PE in the environment. Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops.

Among sulfonylurea products, PE herbicide is also widely used for selective post-emergence control of annual, perennial grasses and broad-leaved weeds in cereals, and is currently recommended for use on some relevant crops in over 30 countries (Singh *et al.,* 2012; Giovanni *et al.,* 2011). Due its widespread use, it has become a potential water pollutant and presents environmental risk, especially for aquatic organisms, owing to its fairly high water solubility which result in its high mobility. It has been detected in surface and groundwater (Battaglin *et al*., 2001). Phytotoxicity of chlorsulfuron, sulfometuron-methyl and metsulfuron-methyl has been reported for higher plants (Sabater and Carrasco, 1997). Toxicity of tri-sulfuron on aquatic organisms has been reported earlier (Baghfalaki *et al*., 2012). ***However, the toxic potential of PE on fresh water teleost O.mossambicus is lacking.***

Together taken the literature review, lab studies and field studies of different agrochemicals have proven a negative correlation of toxic effect of it to the growth of fish. With this we hypothesized what will happen when different groups of agrochemicals are acquainted to the fish in aquatic ecosystem. ***To our knowledge, a comprehensive assessment of hormonal alteration (Cortisol, Estradiol, 11-keto testosterone, Thyroid stimulating hormone, Thyroxine (T4), Triidothrionine-T3) and gene expression pattern of major marker involved in shaping the neuroendocrine physiology of O.mossambicus are lacking. Hence, the present study validates the neuroendocrine orchestration on agrochemical (Imidacloprid-IMI, Curzate-CZ, Pyrazosulfuron ethyl-PE and Micronutrient Mixture-MN) exposure with special reference to O.mossambicus.***